Phytochemistry, 1967, Vol. 6, pp. 883 to 887. Pergamon Press Ltd. Printed in England

CHEMISTRY OF THE PODOCARPACEAE—XIII.[1]

CONSTITUENTS OF THE HEARTWOODS OF *PODOCARPUS NIVALIS* HOOK. AND *PODOCARPUS ACUTIFOLIUS* KIRK

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(*Received* 19 *October* 1966)

Abstract—Totarol, podototarin, podocarpic acid, β-sitosterol, and campesterol have been isolated from, or identified as constituents of, the heartwoods of *Podocarpus nivalis* and *P. acutifolius*. The former species also contains methyl podocarpate, the *n*-alkanes with chain length C_{13}, C_{14}, and C_{21}–C_{31} inclusive, and a new bisditerpenoid which has been identified as the monoacetate of podototarin. *P. acutifolius* contains the *n*-alkanes with chain length C_{21}–C_{35}.

CONIFERS belonging to the family Podocarpaceae are represented in New Zealand by species within the three genera, *Podocarpus* (seven species), *Dacrydium* (seven), and *Phyllocladus* (three). These trees are notable for the fact that without exception their leaves contain diterpene hydrocarbons[2] while their heartwoods are usually a rich source of oxygenated diterpenoids.* With the exception of *Dacrydium cupressinum*[4] the heartwoods of the *Dacrydium* species contain non-phenolic bicyclic diterpenoids of the manool type[3] but the heartwood constituents of *Phyllocladus* species are as yet largely uninvestigated.[5] In accord with anatomical features of the wood which resemble those of *Podocarpus* rather than those of *Dacrydium*,[6] the heartwood constituents of *D. cupressinum* show little affinity to diterpenoids of other *Dacrydium* species, but are of the phenolic tricyclic type typified by podocarpic acid and totarol which are found in *Podocarpus* species.[7] The heartwood or bled resin constituents of five of the seven New Zealand *Podocarpus* species have hitherto been investigated and the results are summarized in Table 1. The heartwoods of the remaining two, *P. nivalis* Hook. and *P. acutifolius* Kirk, have now been examined and their phenolic diterpenoid constituents also found to be of the podocarpic acid—totarol type. Previous investigation of these species has been restricted to the essential oils of the leaves, that from *P. nivalis* containing α-pinene, myrcene, an unidentified sesquiterpene,[8] and the diterpenes, phyllocladene, isophyllocladene, kaurene, and hibaene (cupressene),[2,8] while isokaurene and rimuene have been identified in the oil from *P. acutifolius*.[2]

* The constituents of New Zealand species of the Podocarpaceae have recently been compiled in a phytochemical register.[3]

[1] Part XII. C. R. BENNETT and R. C. CAMBIE, *Tetrahedron* **23**, 927 (1967).
[2] R. T. APLIN, R. C. CAMBIE and P. S. RUTLEDGE, *Phytochem.* **2** 205 (1963); R. T. APLIN and R. C. CAMBIE, *New Zealand J. Sci.* **7** 258 (1964).
[3] S. G. BROOKER, R. C. CAMBIE and M. A. JAMES, *Trans. Roy. Soc. New Zealand, Gen.* **1**, 205 (1966).
[4] C. W. BRANDT and B. R. THOMAS, *New Zealand J. Sci. Tech.* **33B**, 30 (1951).
[5] S. K. ADHIKARI, R. A. BELL and W. E. HARVEY, *J. Chem. Soc.* 2829 (1962).
[6] H. R. ORMAN and J. S. REID, *New Zealand J. Forestry* **5**, 215 (1946).
[7] C. W. BRANDT and B. R. THOMAS, *Nature* **170**, 1018 (1952).
[8] J. MURRAY, *J. Appl. Chem.* **10**, 366 (1960).

TABLE 1. PHENOLIC DITERPENOID CONSTITUENTS OF NEW ZEALAND *Podocarpus* HEARTWOODS

Section and species	Ferruginol	6,7-Dehydroferruginol	Sugiol	Xanthoperol	Podocarpic acid	Methyl podocarpate	Pododacric acid	Totarol	16-Hydroxytotarol	16-Oxototarol	16-Carboxytotarol	Podototarin	Podototarin monoacetate	Refs.
Sect. Dacrycarpus														
P. dacrydioides A. Rich.	+	+	+	+	+	+	+							9
Sect. Eupodocarpus														
P. acutifolius Kirk					+			+				+		*
P. hallii Kirk			+		+			+	+	+	+			10
P. nivalis Hook.					+	+		+				+	+	*
P. totara G. Benn.			+		+	+	+	+	+			+		11
Sect. Stachycarpus														
P. ferrugineus G. Benn.†	+	+	+											12, 13
P. spicatus R. Br.														14

* Present investigation.
† Only the constituents of the bled resin of this tree have been investigated.

Isolation of the constituents from each heartwood was carried out by methods previously detailed for the investigation of other *Podocarpus* species[9, 10, 11, 14] in which a methanolic extract was mixed with Celite to provide a solid support and this then re-extracted with solvents of increasing polarity. As was the case for *P. totara*[11] and *P. hallii*,[10] totarol comprised the major diterpenoid of both *P. nivalis* (0·41 per cent) and *P. acutifolius* (0·09 per cent), and it was isolated from the neutral or phenolic fractions of light petroleum extracts of each tree. The related bisditerpenoid podototarin was also obtained from the phenolic fractions of ether extracts of each species in yields of 0·09 per cent and 0·002 per cent respectively. Podocarpic acid was identified by comparative thin-layer chromatography in the phenolic fraction of the light petroleum extract of *P. nivalis* and was isolated as methyl podocarpate by methylation of the acidic fraction of the ether extract with diazomethane followed by alumina chromatography. Methyl podocarpate itself was also identified in the phenolic and neutral fractions of the ether extract of *P. nivalis* by comparative thin-layer chromatography but was not isolated.

Chromatography of the neutral fraction of the ether extract from *P. nivalis* on alumina gave, from light petroleum—benzene eluates, a new compound (0·031 per cent), $C_{42}H_{60}O_3$, m.p. 237–238°, which was identified as podototarin monoacetate from the following evidence.

[9] L. H. BRIGGS, R. C. CAMBIE, R. N. SEELYE and A. D. WARTH, *Tetrahedron* **7**, 270 (1959).
[10] R. C. CAMBIE, W. R. J. SIMPSON and L. D. COLEBROOK, *Tetrahedron* **19**, 209 (1963).
[11] R. C. CAMBIE and L. N. MANDER, *Tetrahedron* **18**, 465 (1962).
[12] C. W. BRANDT and L. G. NEUBAUER, *J. Chem. Soc.* 1031 (1939); 683 (1940).
[13] L. H. BRIGGS and R. C. CAMBIE, *Tetrahedron* **8**, 356 (1960); J-B. BREDENBERG, *Acta Chem. Scand.* **11**, 932 (1957).
[14] L. H. BRIGGS, R. C. CAMBIE and J. L. HOARE, *Tetrahedron* **7**, 262 (1959).

Carbonyl absorption in the i.r. spectrum at 1764 cm^{-1} was consistent with that of aryl acetates (cf. ν_{max} 1764 cm^{-1} for totaryl acetate and podototarin diacetate[10]) while a sharp peak at 3525 cm^{-1} was indicative of a sterically hindered but non-intermolecularly bonded phenolic group similar to that of podototarin ($\nu_{max}^{CHCl_3}$ 3530 cm^{-1}). The u.v. spectrum possessed absorption maxima at 285 nm and 245 nm and clearly showed that the compound was a bisditerpenoid, the latter peak being indicative of a biphenylic system.[10, 15] Acetylation gave podototarin diacetate identical with an authentic sample.

In contrast to the NMR spectra of the other naturally occurring bisditerpenoids, podototarin[10] and macrophyllic acid,[15] the spectrum of podototarin monoacetate was not that of a symmetrical molecule whose proton count corresponded to half of the molecule. While signals at 0·93 and 0·95 δ (12 protons) corresponding to the C_{15}- and C_{16}-*gem*-dimethyl groups at C_4 and C_4' were superimposed, those of the C_{17}-angular methyl groups (6 protons) were distinct (1·21 and 1·22 δ) and the isopropyl methyl groups gave rise to two sets of two doublets at 1·25, 1·32, 1·36 and 1·37 δ, each with coupling constant, $J = 7{\cdot}0$ c/s. Such a pattern arises from two isopropyl groups each with different chemical shifts and, because of the asymmetry of the molecule, each differentially shielded in a manner similar to that of the isopropyl groups of other totaryl and podotarin derivatives.[10, 15] The signal assigned to the acetate group was unusual in two respects. Firstly, it appeared at 1·79 δ, considerably upfield from the normal position for an aryl acetate (cf. 2·27 δ for the C_3-acetate group of estradiol diacetate[16]) and this marked diamagnetic shift is attributed to the acetate group lying in the cone of shielding of the non-acetyl bearing aromatic ring of the non-planar biphenylic system.[17, 18] Secondly, the acetate signal appeared as a doublet with unequal arms separated by 5 c/s. This splitting which was temperature dependent is attributed to the existence of two stable conformations about the central bond of the biphenylic system as a result of the acetyl group acting as a rotational barrier to interconversion.[18] This phenomenon is also observed in other derivatives of podototarin and will be the subject of a further communication. Further evidence of conformational effects associated with the lack of planarity of the biphenylic system was apparent with the signal corresponding to the aromatic protons of the monoacetate which appeared at 7·05 δ as a doublet with arms separated by 2·0 c/s. The remainder of the spectrum was unexceptional, a sharp phenolic singlet which exchanged with deuterium oxide, appearing at 5·07 δ and an isopropyl methine proton multiplet ($J = 7{\cdot}0$ c/s) occurring at 3·37 δ. On analogy with deshielding effects observed for octahydrophenanthrenes[19] a multiplet at 2·24 δ could be assigned to the C_1-equatorial protons which are deshielded as a result of their close proximity to the C_{11}-aromatic protons.

In addition to phenolic diterpenoids, a fraction was isolated from the neutral fraction of the light petroleum extracts of both *P. nivalis* and *P. acutifolius* whose properties corresponded to those of β-sitosterol. Like most samples of β-sitosterol which have been isolated from plant sources the present samples (0·01 per cent from *P. nivalis* and 0·02 per cent from

[15] S. M. Bocks, R. C. Cambie and T. Takahashi, *Tetrahedron* **19**, 1109 (1963).
[16] N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry*, p. 11. Holden-Day, San Francisco (1964).
[17] Cf. D. R. Dalton, M. P. Cava and K. T. Buck, *Tetrahedron Letters* No. 31, 2687 (1965).
[18] Cf. The diamagnetic shielding of the central methylene groups which lie directly over the aromatic ring in 1,4-polymethylene benzenes [J. S. Waugh and R. W. Fessenden, *J. Am. Chem. Soc.* **79**, 846 (1957).] and of the 2,2'-methyl groups of 2,2',4,4'-tetramethylbiphenyl but not the 4,4'-methyl groups [N. S. Bhacca, L. F. Johnson and J. N. Shoolery, NMR Spectra Catalog., Varian Associates Vol. 2, Spectrum No. 654 (1962)].
[19] W. Nagata, T. Terasawa and K. Tori, *J. Am. Chem. Soc.* **86**, 3746 (1964).

P. acutifolius) contained an impurity[20] which was identified as campesterol by gas–liquid chromatographic comparison with homogeneous samples of these sterols.[21] Light petroleum extracts of both heartwoods also contained alkane fractions. Temperature programmed gas–liquid chromatography showed that the wax from *P. nivalis* (0·038 per cent) contained the *n*-alkanes with chain length C_{13}, C_{14}, and C_{21}–C_{31} inclusive, while that from *P. acutifolius* (0·054 per cent) contained *n*-alkanes with chain length C_{21}–C_{35} inclusive.

EXPERIMENTAL

Microanalyses were by Dr. A. D. Campbell and his associates, University of Otago, New Zealand. I.r. spectra were measured with Perkin-Elmer infracord and 237 instruments and u.v. spectra were determined for EtOH solutions with a Perkin-Elmer 137 u.v. spectrophotometer. NMR spectra were determined in deuterochloroform with a Varian A60 spectrometer using tetramethylsilane as internal reference.

Chromatography of extractive fractions on alumina (P. Spence and Co., type H) and paper chromatography were carried out as described previously.[9–11] Thin-layer chromatography (TLC) was carried out on plates of silica gel G with ethyl acetate as solvent for "acid fractions", chloroform for "phenolic fractions", and benzene–chloroform (1:1) for "neutral fractions", and the plates were developed with iodine vapour.

The analysis of alkanes by gas–liquid chromatography (GLC) was carried out on a Pye-Argon panchromatograph with stationary phases of 1% apiezon L and 1% SE-30 and a temperature variation at the rate of 5°/min over the range 70–350°.

Heartwood Extractions

The finely ground heartwoods of *Podocarpus nivalis** (1·05 kg) and *P. acutifolius*† (6·78 kg) were each extracted (Soxhlet) with methanol for 24 hr and 32 hr, respectively. Solvent was removed from the extracts *in vacuo* and the concentrates were mixed with Celite and air-dried to yield dark brown friable powders. These were then successively extracted (Soxhlet) for 24 hr with light petroleum, ether, and ethyl acetate.

The residues from the light petroleum and ether extracts (13·25 g and 13·44 g, respectively, for *P. nivalis* and 25·2 g and 2·54 g, respectively, for *P. acutifolius*) were dissolved in ether and fractionated between saturated sodium hydrogen carbonate and 10% sodium hydroxide solutions to yield "acidic", "phenolic", and "neutral" fractions.

P. nivalis

Light Petroleum Extract

Comparative TLC showed the presence of totarol (R_f 0·97, yellow spot) and podocarpic acid (R_f 0·67, orange-yellow spot) in the "acidic" (0·6 g) and "phenolic" (1·5 g) fractions, and of podototarin (R_f 0·05, fluorescence under u.v. light) in the "phenolic" fraction.

The neutral fraction (9·8 g) was chromatographed on alumina (350 g). Initial light petroleum eluates contained *n*-alkanes, GLC of which showed the presence of compounds with chain length (percentage composition in brackets) C_{13} (5·2), C_{14} (3·7), C_{21} (6·0), C_{22} (2·7), C_{23} (2·9), C_{24} (2·5), C_{25} (2·5), C_{26} (2·4), C_{27} (7·3), C_{28} (3·5), C_{29} (33·0), C_{30} (4·6), and C_{31} (25·0).

Concentration of the benzene–ether eluates and recrystallization of the residue from ethanol gave "β-sitosterol" (100 mg) as plates, m.p. and mixed m.p. 132·5–133° (identical i.r. spectra); acetate, m.p. and mixed m.p. 122–124°. Comparative GLC using a 4 ft column of 3% SE-30 on chromosorb Z (100–120 mesh) at 235°[21] showed that the material was composed of β-sitosterol and campesterol in the ratio 93:7.

Comparative TLC of the remaining fractions from column chromatography indicated the presence of totarol in ether and ethyl acetate eluates and of methyl podocarpate (R_f 0·95, orange-red spot) in ethyl acetate–acetone eluates.

* Leaf and bark samples of the tree collected in May, 1957, from National Park, Tongariro, were lodged in the Herbarium of the Auckland Institute and Museum under the No. 46,496.

† A.I.M. Herbarium No. 104,585 for leaf samples of the tree collected from Ngahere, Westland, in February, 1965.

[20] J. A. STEELE and E. MOSETTIG, *J. Org. Chem.* **28**, 571 (1963); M. J. THOMPSON, S. J. LOULOUDES, W. E. ROBBINS, J. A. WATERS, J. A. STEELE and E. MOSETTIG, *Biochem. Biophys. Res. Commun.* **9**, 113 (1962); M. CASTLE, G. BLONDIN and W. R. NES, *J. Am. Chem. Soc.* **85**, 3306 (1963); M. J. THOMPSON, W. E. ROBBINS and G. L. BAKER, *Steroids* **2**, 505 (1963); G. I. FUJIMOTO and A. E. JACOBSON, *J. Org. Chem.* **29**, 3377 (1964).

[21] Cf. I. NISHIOKA, N. IKEKAWA, A. YAGI, T. KAWASAKI and T. TSUKAMOTO, *Chem. Pharm. Bull.* (*Tokyo*) **13**, 379 (1965).

Ether Exract

Comparative paper chromatography of the "acidic" fraction (1·45 g) showed the presence of podocarpic acid and an unidentified compound (R_f 0·26, red spot). The fraction was methylated with an excess of an ethereal solution of diazomethane and the product was chromatographed on deactivated alumina. Methyl-podocarpate was contained in fractions eluted with benzene and benzene–ether mixtures.

Alumina chromatography of the "phenolic" fraction (0·8 g) yielded podototarin (4 mg) from the initial benzene–ether eluate. Recrystallization from benzene–light petroleum gave needles, m.p. and mixed m.p. 216–218·5° (identical i.r. spectra). Totarol and methyl podocarpate were identified by TLC in later benzene–ether and acetone–methanol eluates, respectively.

The "neutral" fraction (8·13 g) was chromatographed from benzene on alumina. The benzene–light petroleum (1:9) eluate yielded crystals of podototarin monoacetate (330 mg), m.p. 228–232°, while benzene–light petroleum (1:1) eluates gave an oil which afforded mixed crystals of podototarin and totarol. Podototarin (19 mg) was separated by recrystallization from chloroform–methanol to give needles, m.p. and mixed m.p. 222·5–224° (identical i.r. spectra). Concentration of the benzene and benzene–ether eluates gave totarol (3·82 g) as prisms, m.p. and mixed m.p. 126–128° (identical u.v. and i.r. spectra). Rechromatography of succeeding eluates on alumina gave further totarol (450 mg) and podototarin (19 mg).

Podototarin Monoacetate

After recrystallization from light petroleum *podototarin monoacetate* was obtained as needles, m.p. 237–238° (Found: C, 82·2; H, 10·1. $C_{42}H_{60}O_3$ required: C, 82·3; H, 9·9%), λ_{max} 285 (log ϵ 3·32), 245 (log ϵ 3·90), and 222 nm (log ϵ 4·38), ν_{max} 3540 (phenolic OH), 1760 (aryl acetate), 1380, 1360 (*gem*-dimethyl) and 1190 cm^{-1} (aryl acetate).

The compound (27 mg) was heated to 100° in the presence of acetic anhydride (1·1 ml) and pyridine (2 drops) for 3 hr. Crystallization of the product from ethanol gave needles (24 mg) of podototarin diacetate, m.p. and mixed m.p. 232–234° (identical u.v. and i.r. spectra). Attempts to prepare the monoacetate by partial acetylation of podototarin were unsuccessful.

P. acutifolius

Light Petroleum Extract

TLC of the "acidic" fraction (1·46 g) showed that its major constituents were podocarpic acid, totarol, and an unidentified compound (R_f 0·39). The fraction was methylated with an excess of an ethereal solution of diazomethane and the product was chromatographed on silica gel. Totarol (41 mg) was obtained from light petroleum–benzene (1:1) eluates while methyl podocarpate and methyl-*O*-methylpodocarpate were identified in subsequent fractions by comparative TLC.

Alumina chromatography of the "phenolic" fraction (7·05 g) yielded totarol (290 mg) from benzene eluates but the bulk of material was irreversibly absorbed.

Chromatography of the "neutral" fraction (16·6 g) on deactivated alumina (500 g) afforded from initial light petroleum eluates an *n*-alkane fraction, GLC of which showed the presence of compounds with chain length (percentage composition in brackets) C_{21} (3·1), C_{22} (5·7), C_{23} (5·6), C_{24} (7·7), C_{25} (9·5), C_{26} (9·2), C_{27} (9·2), C_{28} (11·5), C_{29} (8·7), C_{30} (5·9), C_{31} (6·7), C_{32} (4·5), C_{33} (4·5), C_{34} (4·7), and C_{35} (3·5). Concentration of later light petroleum eluates gave podototarin (140 mg) as needles, m.p. and mixed m.p. 218–220° (identical i.r. spectra). Totarol (5·77 g), m.p. and mixed m.p. 127°, was obtained from benzene eluates, and was purified by vacuum sublimation. Elution of the column with more polar solvents gave a series of oils which after further chromatography afforded "β-sitosterol" (1·39 g) as plates, m.p. and mixed m.p. 133–135°, from ether eluates. GLC of the material as for *P. nivalis* showed the presence of β-sitosterol and campesterol in the ratio 91:9.

Ether Extract

TLC showed the presence of podocarpic acid in the "acidic" fraction (440 mg) and of methyl podocarpate and methyl-*O*-methylpodocarpate after the fraction had been treated with an excess of an ethereal solution of diazomethane.

Chromatography of the "phenolic" fraction (1·16 g) on deactivated alumina (100 g) gave podototarin (11 mg) and totarol (47 mg) from light petroleum and benzene eluates, respectively. TLC also showed the presence of these compounds in the "neutral" fraction (0·66 g) and both podototarin (17 mg) and totarol (80 mg) were isolated by alumina chromatagraphy.

Acknowledgements—The authors are grateful to Mr. P. Allan, New Zealand Forest Service, Westland, for supplying the wood of *P. acutifolius* and to Dr. C. J. W. Brooks and Miss P.Pellitt, University of Glasgow, for the temperature-programmed GLC analysis of *n*-alkanes.